CLAIMS

We claim:

1. A multi-domain fusion protein expression cassette, comprising a promoter operably linked to a nucleic acid molecule which is expressed as an insoluble protein, wherein said nucleic acid molecule encodes a polypeptide comprising the structure (cationic peptide)-[(cleavage site)-(cationic peptide)]$_n$, wherein n is an integer having a value between 1 and 100 and said cationic peptide has antimicrobial activity.

2. The expression cassette according to claim 1 wherein said nucleic acid molecule also encodes a carrier protein.

3. The expression cassette according to claim 1 wherein n has a value of between 5 and 40.

4. The expression cassette according to claim 1 wherein said cleavage site can be cleaved by low pH or by a reagent selected from the group consisting of cyanogen bromide, 2-(2-nitrophenylsulphenyl)-3-methyl-3'-bromoindolenine, hydroxylamine, *o*-iodosobenzoic acid, Factor Xa, thrombin, enterokinase, collagenase, *Staphylococcus aureus* V8 protease, endoproteinase Arg-C, and trypsin.

5. The expression cassette according to claim 1 wherein said nucleic acid molecule encodes a fusion protein comprising (a) a carrier protein, (b) an anionic spacer peptide component having at least one peptide with the structure (cleavage site)-(anionic spacer peptide), and (c) a cationic peptide component having at least peptide with the structure (cleavage site)-(cationic peptide) wherein the cleavage site can be on either side of the anionic spacer peptide or cationic peptide, and elements (a), (b), and (c) can be in any order and or number.

6. The multi-domain fusion protein expression cassette of claim 5 wherein said carrier protein is located at the N-terminus of said fusion protein.

7. The expression cassette according to claim 5 wherein said anionic spacer lacks a cysteine residue.

8. The expression cassette of claim 1 wherein said fusion protein comprises from 2 to 40 cationic peptides.

9. The expression cassette according to claim 8 wherein said fusion protein comprises from 3 to 15 cationic peptides.

10. The expression cassette according to claim 5 wherein the number of anionic spacer peptides greater than or the same as the number of cationic peptides.

11. The expression cassette according to claim 5 wherein the number of anionic spacer peptides is less than the number of cationic peptides.

12. The expression cassette according to claim 2 wherein said carrier protein is less than 100 amino acid residues in length.

13. The expression cassette according to claim 2 wherein said carrier protein is a truncated cellulose binding domain of less than 100 amino acids.

14. The expression cassette according to claim 1 wherein said nucleic acid molecule encodes a fusion protein comprising (a) an anionic spacer peptide component having at least one peptide with the structure (cleavage site)-(anionic spacer peptide), and (b) a cationic peptide component having at least peptide with the structure (cleavage site)-(cationic peptide), wherein the cumulative charge of said anionic spacer peptide component reduces the cumulative charge of said cationic peptide component.

15. The expression cassette according to claim 1 wherein said promoter is selected from the group consisting of *lacP* promoter, *tacP* promoter, *trcP* promoter, *srpP* promoter, SP6 promoter, T7 promoter, *araP* promoter, *trpP* promoter, and λ promoter.

16. A recombinant host cell comprising the expression cassette according to claim 1.

17. The recombinant host cell of claim 16 wherein said host cell is a yeast, fungi, bacterial or plant cell.

18. The recombinant host cell of claim 17 wherein said bacterial host cell is *Escherichia coli*.

19. A polypeptide encoded by the expression cassette according to claim 1.

20. A method of producing fusion proteins that contain a cationic peptide, comprising: (a) culturing the recombinant host cell of claim 15 under conditions and for a time sufficient to produce said fusion protein.

21. The method according to claim 19, further comprising the step of isolating said fusion protein.

22. The method according to claim 20, further comprising the step of cleaving said polypeptide by treating said fusion protein with low pH or with a reagent selected from the group consisting of cyanogen bromide, 2-(2-nitrophenylsulphenyl)-3-methyl-3'-bromoindolenine, hydroxylamine, *o*-iodosobenzoic acid, Factor Xa, thrombin, enterokinase, collagenase, *Staphylococcus aureus* V8 protease, endoproteinase Arg-C, and trypsin.

23. The method according to claim 21, further comprising the step of purifying said cationic peptides by applying said cleaved polypeptide to an anion exchange chromatography resin.

24. The method according to claim 23 wherein said anion exchange column is charged with a base, and washed with water prior to loading the column with said cationic peptide.

25. The method according to claim 23 wherein said column is equilibrated with water and up to about 8 M urea.

26. The method according to claim 23 wherein said cationic peptide is in a solubilized in a solution comprising up to about 8 M urea.

27. The method according to claim 23 wherein said cationic peptide is solubilized in a solution comprising a mild organic solvent.

28. The method according to claim 27 wherein said mild organic solvent is methanol, ethanol, or acetonitrile.

add C5

add E8